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In vitro Evaluation of Antimitotic, Antiproliferative, DNA fragmentation and Anticancer activity of Chloroform and Ethanol extracts of *Rivea hypocrateriformis*

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ABSTRACT

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Objective: The plant *Rivea hypocrateriformis* (RH) has numerous therapeutic utility in folk medicine having antidiabetic, antidepressant, analgesic as well as pregnancy irruption and anticancer properties. This led us to carry out the evaluation of plant for antimitotic, antiproliferative and cytotoxicity studies. **Materials and Method:** The dried aerial parts of RH were successively extracted with petroleum ether, chloroform, ethanol and water. All extracts are subjected to *in vitro* Antimitotic and Antiproliferative assay by *Allium cepa* root inhibition and yeast model. The successive chloroform, SCH and ethanol extract, SEE was subjected to *in vitro* anticancer activity by SRB assay MCF-7, HOP-62, MOLT-4, HCT-15 and PRO cell lines. **Results:** The SCH and SEE shows significant antimitotic and antiproliferative activity. The mitotic index was found to be 12.14 and 14.24 mg/mL respectively, which was near to standard, Methotrexate 11.39. The IC₅₀ value of antiproliferative assay was found to be 47.88 to 27.12 mg/mL for SCH and SEE respectively. **Conclusions:** Based on these results, it is concluded that RH may be the good candidate for the treatment of cancer as SCH and SEE are cytotoxic against various cell line in SRB assay.

1. Introduction

Plant drugs have a long history in both traditional and modern societies as herbal remedies or crude drugs. A high number of new drugs derived from plant secondary metabolites have been applied towardsly in the treatment and/or prevention of cancer. Since 1990, there has been a 22% increase in cancer incidence and mortality, with over 10 million new cases and over 6 million deaths worldwide in 2000 (excluding non-melanoma skin cancers). Important progress has been made in cancer chemotherapy, a considerable portion of which can be attributed to plant-derived drugs [1]. Drug discovery from plants still provides important new

drug leads, many of which are approved or undergo trials for clinical uses against cancer, malaria, Alzheimer disease, HIV/AIDS, pulmonary pathologies and other diseases [2]

Rivea hypocrateriformis (convulvaceae) is climbing shrub found all over India. Even though the plant is known for a large number of biological activities such as antidiabetic, antiimplantation, in treatment of burning & piles, pregnancy irruption, antidepressant, anticancer analgesic, antioxidant and hepatoprotective properties [3–5]. Phytochemically it contains gallic acid, lupeol and amino acid [5, 6]. Literature survey reveals lack of any scientific cytotoxic investigation of this plant. Hence in present study the plant was subjected to evaluation of antimitotic, antiproliferative and cytotoxicity on cancer cell lines.

2. Materials and methods

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2.1 Herbal material and extraction

R.hypocrateriformis collected in the month of August to September, 2009 from Amravati District, Maharashtra and it is authenticated by Prof. Dr. Bhowagaokar (Taxonomist), Botany Department, VIHS Amravati, Maharashtra. Plant materials successively extract, hot continuous extraction with petroleum ether, chloroform and ethanol. The solvent was evaporated to dryness under pressure using rotary flash evaporator to obtain crude extracts.

2.2 Antimitotic activity

A modified method described by Murthy *et al* [7] was used for evaluation of antimitotic activity using *Allium cepa* root. *Allium cepa* bulbs were sprouted in water for 48h at room temperature. The bulbs that developed uniform root were selected for the study. These roots were dipped in the extract (10 mg/mL) for 3h. Water was used for dilution as well as a blank and Methothrexate was used as a standard for study. After 3h, the root tips were fixed in the fixing solution of acetic acid and alcohol (1:3). Squash preparation was made by staining with acetocarmine stain. The mitotic index was calculated by following formula:

$$\text{Mitotic index} = \frac{\text{Number of dividing cell}}{\text{Total number of cells}} \times 100\%$$

2.3 Antiproliferative activity

Antiproliferative study was evaluated by yeast *Saccharocymes cerevisiae* model according to the previously reported method of Julian *et al* [8].

2.3.1 Preparation of Yeast inoculums

The yeast was inoculated with sterilized potato dextrose broth and incubated at 37 °C for 24hrs, referred as seeded broth. The seeded broth diluted with sterilized distilled water, in order to get 25.4×10^4 cells (average).

2.3.2 Cell viability count

For cell viability count solution containing 2.5 mL of potato dextrose broth and 0.5 mL of yeast inoculums were prepared in four separate test tubes. In the first and second tubes, 1 mL of SCH and SEE mixed while third tubes standard Methothrexate was added. The fourth tubes kept control without extract. All tubes were incubated at 37 °C. In the above cell suspension, 0.1% methylene blue dye was added in all tubes and they were observed under low power microscope. The no. of viable cells, those does not stain and look transparent with oval shape while dead cells get stained and appeared blue in color were counted

in 16 chambers of hemocytometer and the average no. of cell was calculated.

2.4 DNA fragmentation Assay

DNA fragmentation assay performed by the method of Bicas *et al* [9]. Briefly, 0.1 mL of extract mixed with 2.5 mL potato dextrose broth and 0.5 mL of yeast inoculums. Cell suspension was incubated for 24 hours at 37 °C. Sample was chromatographed at 50 V in a gel electrophoresis by using Hind III marker [9].

2.5 Cytotoxicity study by SRB assay

The Sulforhodamine B, SRB method was used as described previously by Ashidi *et al* [11]. Briefly a cell concentration of 4×10^3 cells/mL was used and vincreastine sulphate and Adriamycin was used as a positive control.

3 Results

3.1 Antimitotic activity

The results of antimitotic activity revealed that SCH & SEE both shows good inhibition of meristamatic cell in different stages of cell cycle (Fig 1) and their mitotic index were found to be 14.24 and 12.14 respectively which was close to standard, Methothrexate (Table 1).

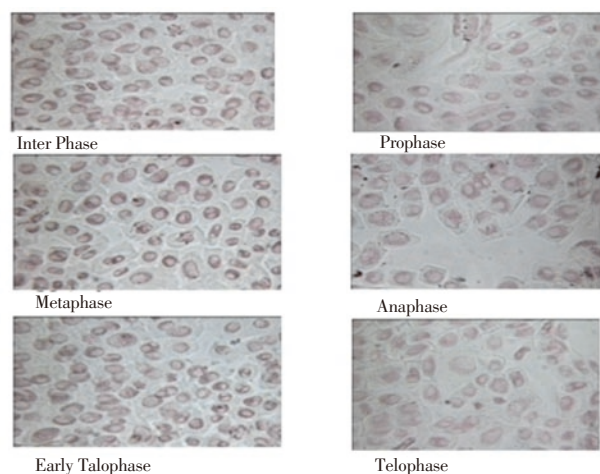


Figure 1 Stages of meristamatic cell division

3.2 Antiproliferative assay

Cell type antiproliferative specificity is observed in plant extracts. This specificity of plant extracts is likely to be due to the presence of different classes of compounds in the extract, as it has been documented in the case of known classes

of compounds [1]. Antiproliferative assay was evaluated by *Saccharomyces cerevisiae* yeast model. In this study both extracts shows good inhibition of yeast cell growth. The viable cell count for SCH and SEE were found to be 282×10^3 cells/mL and 283×10^3 cells/mL respectively. These value decrease dose dependently with increase in concentration of the extracts with IC_{50} value estimated 47.88 and 39.15 mg/mL for SCH and SEE respectively (Table 1).

Table 1

Mitotic index and IC_{50} value of Antiproliferative assay

Extract	Mitotic Index	IC_{50} (mg/mL)
SEE	12.14±0.37	27.12
SCH	14.24±0.14	47.88
Methothrexate	11.39±0.19	17.89

3.3 DNA Fragmentation Assay

DNA fragmentation assay confirmed the antiproliferative effect of extracts. A cancer cell is a mutant human cell that differs from normal cell only in the rapid growth of cell. The DNA of the rapidly multiplying, cancerous cells is more exposed as compared to normal cells. Thus, the cell DNA is one of the targets for the treatment of cancer. Breakdown of DNA molecule is one of the sign of inhibition of DNA replication, which may be due to the inhibition of topoisomerase, key enzymes in DNA replication (Fig 2a & 2b).

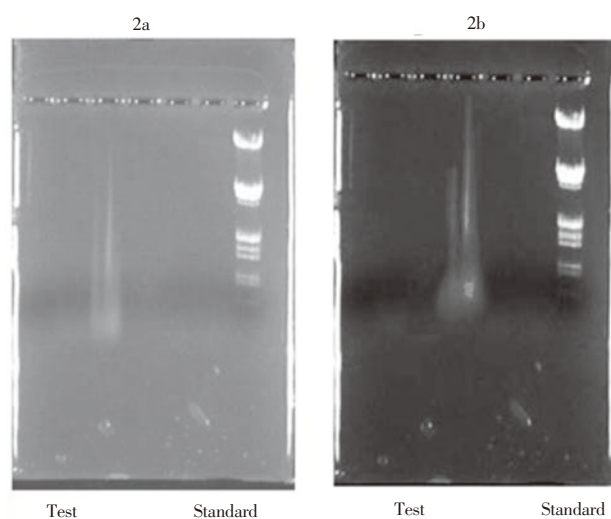


Figure 2 DNA fragmentation

3.4 Cytotoxicity study

The results of preliminary screening for cytotoxicity of the extracts are summarised in Table 2. In the US NCI plant screening program, a crude extract is generally considered to have in vitro cytotoxic activity if the IC_{50} value below $30 \mu\text{g/mL}$ and for pure compounds, below $4 \mu\text{g/mL}$ [12]. Results shows that SCH and SEE both have good anticancer activity

against Leukemia, MOLT-4 while moderate activity on Breast cancer cell line, MCF-7. Both extracts shows some effect on Colon, HCT-15 and Lungs cancer cell line, HOP-62 but ineffective against Prostate cancer cell line, PRO.

Table 2

IC_{50} value of SRB assay on different cell lines

Cell lines	Concentration ($\mu\text{g/mL}$)			
	SEE	SCH	Vincristine	Adriamycin
MCF-7	19	20	10	10
HCT-15	>80	>80	10.2	09
MOLT-4	16	18	14	12
HOP-62	28	32	20	08
PRO	>80	>80	>80	08

4. Discussion

Despite of potential traditional or tribal used of RH very few scientific work is reported. To the best of our knowledge we are the first which exploring the anticancer properties of this plant. Cancer is a disease recognised by seven hallmarks: unlimited growth of abnormal cells, self sufficiency in growth signals, insensitivity to growth inhibitors, evasion of apoptosis, sustained angiogenesis, inflammatory microenvironment, and eventually tissue invasion and metastasis [12]. A survey of the literature revealed that no studies on the anticancer activity of extracts of plants was carried out hence above mentioned, had been undertaken on the five human cancer cell lines. It is known that different cell lines might exhibit different sensitivities towards an anticancer compound, so the use of more than one cell line is therefore considered necessary in the detection of anticancer compounds. From the data obtained, it was observed that both SCH and SEE extracts highly effective against MOLT-4 and MCF-7 where as SEE have moderate effect on HOP-62 comparable to the standards vincristine and adriamycin. Phytochemically SCH & SEE was rich in polyphenolic and triterpenoid constituents. The major polyphenolic and triterpenoid in SEE was identified as gallic acid lupeol [5]. Polyphenol and triterpenoids are well known for their anticancer activity. These molecules might act as cancer-blocking agents, preventing initiation of the carcinogenic process and as cancer-suppressing agents, inhibiting cancer promotion and progression [13]. It was already reported that gallic acid and lupeol having potent anticancer act by decreasing the mitochondrial membrane potential and intracellular reactive oxygen species along with inhibition of topoisomerase II and angiogenic inhibition [12, 14]. The anticancer property of RH is mainly contributed by gallic acid, lupeol and similar phytoconstituents by one of the above mechanism. Furthermore, DNA fragmentation assay, antimetabolic and antiproliferative study clearly indicates potential of SEE in cancer management. As extracts inhibit

the DNA replication may be due to inhibition of DNA topoisomerase II, a key enzyme in DNA replication. The arrest the cell growth in cell cycle, as they reduces the rate of cell division by preventing the entry of cell into the prophase and subsequent phases which was concluded from the antimitotic and antiproliferative results which may be considered as an alternate mechanism of action of extracts in cell growth inhibition. Thus, it can assume the possible mechanism of the anticancer activity of RH may be due to the presence of triterpenoids and phenolic compounds in the extracts. It may be further concluded that the presense of gallic acid, lupeol and other similar phytoconstituents present in the extract may contribute in cytotoxicity and cancerous cell growth inhibition. Our findings support the reported therapeutic use of this plant as a anticancer agent in the Indian system of medicine. Further experiments are needed, both in vitro and in vivo to obtain more detail mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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